Diffusion and Consumption of Oxygen in the Resting Frog Sartorius Muscle

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ABSTRACT   Adaptations of the method of Takahashi et al. (1966. *J. Gen. Physiol.* 50:317–333) were used to test the validity of the one-dimensional diffusion equation for O_2 in the resting excised frog sartorius muscle. This equation is:

$$D\alpha \frac{\partial^2 P}{\partial x^2} - \alpha \frac{\partial P}{\partial t} = Q,$$

where x is the distance perpendicular to the muscle surface, t is time, $P(x, t)$ is the partial pressure of O_2, D and α are the diffusion coefficient and solubility for O_2 in the tissue, and Q is the rate of O_2 consumption. $P(0, t)$, the time-course of P_{O_2} at one muscle surface, was measured by a micro-oxygen electrode. Transients in the P_{O_2} profile of the muscle were induced by two methods: (a) after an equilibration period, one surface was sealed off by a disc in which the O_2 electrode was embedded; (b) when P_{O_2} at this surface reached a steady state, a step change was made in the P_{O_2} at the other surface. With either method, the agreement between the measured $P(0, t)$ and that predicted by the diffusion equation was excellent, making possible the calculation of D and Q. These two methods yielded statistically indistinguishable results, with the following pooled means (±SEM):

T		$D \times 10^4$ (cm^2/min)	Q_{10}		Q	Q_{10}
°C	n	cm^2/min		n	$\mu l\ O_2/g \cdot min$	
22.8	13	8.54 ± 0.34		22	0.590 ± 0.042	
10	11	6.30 ± 0.30	1.27	16	0.241 ± 0.019	2.01
0	18	4.94 ± 0.16	1.28	14	0.060 ± 0.008	4.04

At each temperature, D was independent of muscle thickness (range, 0.67–1.34 mm). The activation energy (E_A) for diffusion of oxygen in muscle was -3.85 kcal/mol, which closely matches the corresponding value in water. Together with absolute values of D in water taken from the literature, the present data imply that (D_{muscle}/D_{H_2O}) is in the range 0.59–0.69. This value, and that of E_A, are in agreement with the theory of Wang (1954. *J. Am. Chem. Soc.* 76:4755–4763), suggesting that with respect to the diffusion of O_2, to a useful approximation, frog skeletal muscle may be considered simply as a homogeneous protein solution.

INTRODUCTION

In the early stages of an investigation of the kinetics of oxygen consumption in

the excised frog sartorius muscle (Mahler, 1978), it became clear that it was essential to have an accurate description of the time-course of change in the amount of stored oxygen in a stimulated muscle. It has customarily been assumed (Hill, 1928, 1966; Hill, 1940; Kushmerick and Paul, 1976) that such a description can be calculated via the one-dimensional diffusion equation for oxygen, using a value for the diffusion coefficient (D) for oxygen derived from the results of Krogh (1919). The experiments described in this paper were designed to test these assumptions.

The equation in question (Fick's diffusion equation) can be written in the form:

$$D\alpha \frac{\partial^2 P}{\partial x^2}(x, t) - \alpha \frac{\partial P}{\partial t}(x, t) = Q(t),\tag{1}$$

where x is the distance perpendicular to the muscle surface, t is time, P is the partial pressure of oxygen (Po_2), α is the solubility of oxygen in the muscle, D is the diffusion coefficient for oxygen, and Q is the rate of oxygen consumption (Qo_2). D, α, and Q are assumed to be constant throughout the muscle, and Eq. 1 thus describes the diffusion of oxygen through a homogeneous plane sheet in which oxygen is also being consumed at a uniform rate. Despite the heterogeneity of muscle tissue, it can be shown a priori that on a scale of microns, the validity of Eq. 1 in the excised frog sartorius is plausible (cf. Discussion).

Apart from a preliminary report on the results of the present study (Mahler, 1975), the only work that deals directly with the validity of the diffusion equation for oxygen in skeletal muscle appears to be that of Gore and Whalen (1968) and Kawashiro et al. (1975). The classical work of Krogh (1919) also merits a summary here. Krogh maintained an essentially constant Po_2 difference across a sheet of tissue, and measured the amount of O_2 that crossed the tissue over a period of several hours. The average rate of O_2 transport through the tissue, normalized for surface area, thickness, and Po_2 difference, was referred to by Krogh as the "diffusion constant" for oxygen in the tissue; it has since been given the symbol K. It is a straightforward matter to show that if Eq. 1 is valid in such preparations, with Q independent of P (Hill, 1948), K should closely approximate $D\alpha$. However, this method can provide no evidence whether Eq. 1 is in fact valid—that is, whether the movement of oxygen through the tissue has actually taken place by simple diffusion through an essentially homogeneous medium. At 20°C, Krogh's mean value for K in frog skeletal muscle was 1.49 × 10⁻⁵ ml O_2/min·cm·atm.

The first direct test of the validity of the diffusion equation for oxygen in skeletal muscle was that of Gore and Whalen (1968), who used a recessed micro-O_2-electrode to map the intramuscular Po_2, profile of a resting excised frog sartorius at 22°C. For muscles in 25% O_2, their results are consistent with the steady-state solution of Eq. 1, and imply a value for $D\alpha$ of 1.37 × 10⁻⁵ ml/min·cm·atm. For muscles in 10% O_2, however, the results of Gore and Whalen are difficult to interpret. Kawashiro et al. (1975) measured rates of O_2 transport across sheets of rat abdominal muscle at 37°C, under conditions in which the Po_2 difference across the tissue changed very slowly. Their results were consistent with the steady-state solution of Eq. 1, and allowed simultaneous

measurement of both Q and $D\alpha$. The latter had a mean value of 2.53×10^{-5} ml/ min·cm·atm; using the formula postulated by Krogh (1919) for the temperature dependence of K, this is equivalent to 2.16×10^{-5} ml/min·cm·atm at 20°C. The results of Gore and Whalen (1968) and Kawashiro et al. (1975) are consistent with steady-state solutions of Eq. 1 in isolated skeletal muscle, but have not tested its validity in nonsteady states. Moreover, the data reported in these studies, as well as that of Krogh (1919), can accomodate a rather wide range of values for $D\alpha$.

The diffusion coefficient for oxygen (D) does not appear to have been previously directly measured in skeletal muscle. The values given by Hill (1928, 1966) were calculated from the values of K reported by Krogh (1919), and the assumption that $D = K/\alpha$. These values are thus subject to the same uncertainty as Krogh's K (cf. Discussion). In addition, it appears that α has never been directly measured.

In the experiments to be described below, the validity of the one-dimensional diffusion equation (Eq. 1) in the resting excised frog sartorius was tested during periods when the intramuscular Po_2 profile was changing with time. During these periods, the time-course of Po_2 at one surface of the muscle was measured with a Clark-type oxygen electrode; in terms of Eq. 1, this was denoted $P(0, t)$. Transients in the Po_2 profile were induced by interventions which could be expressed analytically as changes in a boundary condition for Eq. 1. These boundary changes were chosen so as to allow exact solutions of Eq. 1; that is, closed form expressions for $P(x, t)$, the predicted transient change in the intramuscular Po_2 profile after the given change in the boundary condition. In particular, expressions could be obtained for $P(0, t)$, the predicted time-course of Po_2 at the muscle surface; a predicted $P(0, t)$ could then be tested by comparison with that actually measured. The results of these experiments support the validity of Eq. 1 on the macroscale in this muscle, and allow direct calculation of the diffusion coefficient D.

MATERIALS AND METHODS

Preparation of Muscle

For most experiments, Northern *Rana pipiens* (Nasco, Fort Atkinson, Wisc.), unsorted for sex, were used; they were kept at 5°C. In a few cases, to obtain muscle of thickness 1.2-1.4 mm, large Southern *R. pipiens* were used; these were kept at room temperature and fed every other day. After dissection, individual sartorii, with split pelvic bones attached, were stored in Ringer solution at 4°C for 2-24 h before use in an experiment. With a few exceptions, the experiments were all performed in the months of December through March.

Oxygen Electrodes

The polarographic oxygen electrodes used were of the basic type described by Fatt (1964). A 25-μm platinum wire and a 500-μm silver wire, lying in parallel, were encased in an epoxy-filled Lucite rod with outer diameter 9.5 mm and length 6-8 cm (Lucite, E. I. DuPont de Nemours & Co., Wilmington, Del.). At one end, the wires were joined to a coaxial cable. During operation, the cable was connected to a battery supplying 0.67 V, and the circuit was closed by covering the other end of the electrode with an electrolyte

solution, held in place by a polyethylene membrane of thickness 12.7 μm. The electrolyte solution employed had the following composition: 50 mM boric acid (H_3BO_3), 50 mM KCl, 21.3 mM NaOH, pH 9. The electrode current was amplified and recorded by a Beckman model 160 gas analyzer (Beckman Instruments, Inc., Fullerton, Calif.) and a Brush model 440 chart recorder (Gould, Inc., Inst. Sys. Div., Cleveland, Ohio). At the conclusion of a day's experiments, the electrode tip was immersed overnight in a NaCl solution, the electrode remaining polarized. Before further use, the platinum surface was swabbed with 15% NH_4OH. The initial stabilization period after cleaning and reassembly was considerably shorter when the electrode had been stored in a polarized condition than when it had been stored unpolarized in air.

Performance Characteristics of Oxygen Electrodes and Recording System

LINEARITY IN TEST GASES   When treated in the above manner, these electrodes showed excellent linearity in test gases at 23, 10, and 0°C, over the Po_2 range 0–700 mm Hg. Before each experiment, the electrode was calibrated at the temperature of the experiment, by using gases whose oxygen tensions spanned the range that would be encountered; experiments were not performed unless the electrode current at the upper limit of this span was within 5% of the theoretical linear value.

TIME RESPONSE   The time response of the entire recording system for Po_2 (oxygen electrode, gas analyzer, and chart recorder) was quantified by measuring its response to a step increase in the Po_2 at the surface of the membrane. Such a change could be readily produced by placing the electrode against a blotted muscle for a short time, then rapidly removing it. When this was done, the system response was adequately approximated by first order kinetics, with τ = 1.8, 2.2, and 3.6 s at 23, 10, and 0°C, respectively. This is fast enough to be considered instantaneous in comparison with the changes in Po_2 which took place at the muscle surface during the experiments.

RATE OF OXYGEN CONSUMPTION   The rate of oxygen consumption by the electrode was at a maximum in 95% O_2 at 23°C, in which case it was calculated to be roughly 3 × 10^{-6} ml O_2/h, ~0.2% as large as the resting Qo_2 observed at 23°C for a 50-mg muscle. This figure was arrived at by calculating the electrode current from the formula given by Fatt (1964), and assuming that, for each molecule of O_2 consumed at the platinum surface, four electrons pass between anode and cathode (Davies, 1962). For other experimental conditions, the Qo_2 of the electrode relative to that of the muscle was even smaller.

Calibration of Electrode with Muscle as the External Medium

When a muscle replaced gas as the medium external to the oxygen electrode, a slightly different calibration curve was used. Within the first few seconds of the initial contact between the electrode and the muscle, a rapid linear change occurred in the electrode current, similar to that reported by Takahashi et al. (1966), but of considerably smaller magnitude. The initial change was usually a decrease, but in ~10% of experiments, a small increase occurred. At 22–23, 10, and 0°C, respectively, the electrode current at the end of this rapid phase averaged 91.5% (± 0.82% SEM; n = 24), 97.0% (± 0.44%; n = 18), and 99.3% (± 0.37%; n = 18) of the current for the chamber gas. These values were independent of the O_2 content of the gas. It was assumed that this rapid decrease in electrode current was caused by the change in the external medium per se, and not by a rapid fall in Po_2 (see below). Accordingly, because the Po_2 at the muscle surface at the time of contact was necessarily the same as that of the chamber gas, it was assumed that the electrode current at the end of the rapid change corresponded to a muscle Po_2 which matched that of the gas. The current corresponding to a Po_2 of zero in the muscle was measured by the method of Takahashi et al. (1966): a moistened glass microscope

cover slip was placed over the upper surface of the muscle; the subsequent time-course of the Po_2 at the lower surface of the muscle was recorded, and the final, constant level was assumed to indicate that the muscle was completely anoxic above the Pt cathode. This current was essentially identical to that observed with a test gas containing zero oxygen (typically 93% N_2, 7% CO_2). Once this fact had been established, the "zero current" in gas, which was measured before each day's experiments, was routinely assumed to be valid for the muscles as well. This current was not susceptible to electrode drift. A calibration curve for muscle constructed in this way was linear over the Po_2 range 0-700 mm Hg.

The cause of the rapid initial change in electrode current after contact with a muscle is not well understood. Goldstick and Fatt (1970) showed that the electrode has a negligible effect on the external medium when it is in a gas; if the gas is replaced by water, however, Gutherman and Goldstick (1974) concluded on theoretical grounds that the oxygen consumption of the electrode should cause the steady-state Po_2 at the outer surface of the membrane to be ~3% less than that in the bulk phase at 20°C, and thus cause a decreased current flow relative to that in the gas. This suggests that the rapid fall in current after the change in external medium from gas to muscle might be due simply to a fall in Po_2. Experiments designed to test this hypothesis did not appear consistent with it (for details, cf. Methods in Mahler, 1976), but neither did they provide an obvious alternative explanation. If a rapid fall in the surface Po_2, caused by the electrode, did in fact occur after contact with the muscle, the electrode calibration curve for muscle as the external medium would then be the same as that determined with test gases, but it would be necessary to correct the Po_2 records for the effect of the electrode. In this context, evidence summarized above would suggest that this effect was the reduction of Po_2 to a fixed percentage of the value that would occur in the absence of the electrode, with this percentage being 91.5, 97.0, or 99.3% at 22-23, 10, and 0°C, respectively. Correcting records on this basis would have exactly the same result as using the calibration procedure for muscle described in the previous paragraph.

As an internal check on calibration, values of (Δcurrent/ΔPo_2) were calculated from steady-state measurements made with method II (see below), and found to agree to within 5-10% with those determined by the method described above.

Chamber for Measurement of PO_2 at Muscle Surface

Fig. 1 is a schematic representation of the chamber constructed for the measurement of Po_2 at the surface of a muscle. The main body of the chamber was a cylinder of Lucite, closed at both ends, with inner diameter 13.1 cm and height 4.6 cm. The top and walls of the cylinder constituted a separate piece, which could be lifted off to mount or remove a muscle. At the center of the chamber floor was a circular hole (diam. 9.5 mm); beneath this was a shaft 2.5 cm long, with inner diameter 9.5 mm. The oxygen electrode was inserted into this shaft, and an attached lever allowed the upper surface of the electrode to be brought flush with the chamber floor, or recessed by 6 mm. A muscle was placed across the top of the shaft, with its length set at the value measured in vivo before dissection. A flow of saturated gas was maintained through the chamber at all times. The entire chamber was immersed in a water bath whose temperature was constant to within 0.1°C. A thermister embedded in the floor of the chamber measured the temperature at the surface of the muscle.

Implementation of Changes in Boundary Conditions for PO_2 at Muscle Surfaces

Muscles were placed in the chamber while the electrode was recessed; because muscle widths were always <9.5 mm, the portion of the muscle lying over the shaft was oxygenated at both surfaces. The chamber was then placed into the water bath. After the

muscle temperature had reached the desired level, a further equilibration period was allowed, to enable the muscle Qo_2 to fall to a constant value characteristic of that temperature, and the Po_2 profile to reach a steady state. At 23, 10, and 0°C, 30, 30, and 60 min, respectively, were allowed for this to occur. Experimental interventions were then begun.

Method I

The object of this method was to abruptly change the boundary condition at the portion of the lower surface of the muscle lying over the shaft. While this surface was exposed to the gas phase, its boundary condition was $P(0, t) = P_0$, where P_0 denotes the Po_2 of the gas phase. At time $t = 0$, the oxygen electrode was raised so that its surface was flush with the floor of the chamber, thereby sealing off the lower surface of the muscle, and presumably changing the boundary condition there to $(\partial P/\partial x) = 0$. The oxygen electrode measured the subsequent $P(0, t)$. This method is similar to that used by Takahashi et al. (1966) in their determination of D and Q in rabbit corneal stroma.
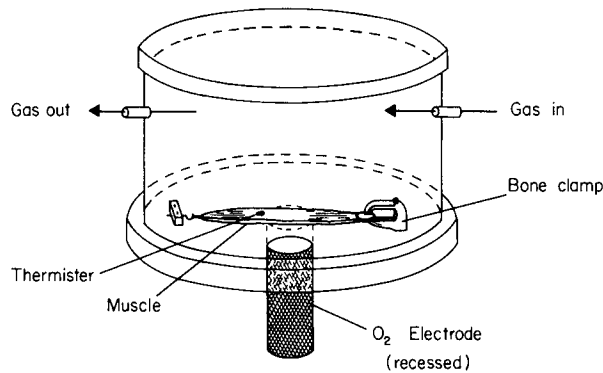

FIGURE 1.   Chamber for measurement of Po_2 at a muscle surface.

Method II.

In a muscle with constant Qo_2, after an intervention of method I above, the Po_2 profile would eventually reach a new steady state, in which oxygen was supplied only at the upper surface. This was evidenced by a constant value of Po_2 at the lower surface, as measured by the electrode. When this condition was met, an abrupt boundary change was made at the upper surface of the muscle at a new time zero, by rapidly changing the Po_2 of the gas in the chamber; this change was 50% complete in ~20 s, and complete in ~3 min. Again, the subsequent time-course of Po_2 at the lower surface of the tissue was measured by the electrode. A somewhat similar method for testing the Fick equation and measuring D was used by Connelly et al. (1953) with frog nerve.

Measurement of Muscle Thickness

The thickness of the muscle at the point above the platinum cathode was measured by focusing a microscope first on the muscle surface, then on the surface of the electrode membrane after the muscle had been removed, and measuring with a micrometer dial the net distance the microscope head had traveled in the process. This was done in the following way. At the conclusion of the experiments, the top of the chamber was removed, and a bracket was attached to the lower portion, allowing it to be affixed to the microscope in place of its stage. During readings, the field was illuminated with a fiber optics light source (type K-150 American Optical Corp., Buffalo, N.Y.), and a flexible

light guide. One eyepiece of the microscope contained a numbered grid, and by using previously determined landmarks, the chamber was positioned so that the platinum cathode, which could not be seen through the muscle, would lie beneath the muscle surface covered by the grid at a magnification of × 215; this was a square of ~300 μm on a side. The chamber was also positioned so that the longitudinal axes of the muscle fibers lay parallel to the X-axis of the grid. Several drops of a suspension of jeweller's rouge in Ringer solution were then placed on the muscle surface, and on the adjoining surface of the electrode; the diameter of the particles was about 5 μm. By touching an absorbent tissue to the fluid next to the muscle, most of the added Ringer solution was then drained off the muscle, leaving a scattering of particles. At a magnification of × 215, the microscope was then focused on each of 5–10 particles, whose positions along the Y-axis of the grid spanned its entire range; the height of the microscope head was recorded in each instance via a fine focus knob graduated to 1 μm. Readings were found to be independent of the particle position along the X-axis. The muscle was then removed, and the focusing procedure was repeated on the surface of the electrode membrane; here, only one reading was taken, over the platinum cathode, which was now visible. The position of the cathode on the Y-axis of the grid determined which of the readings taken from the muscle surface was to be used; if necessary, linear interpolation between readings from adjoining points was employed. The error in measurements of tissue thickness over the cathode by this method was probably always <~15 μm, while the measured values ranged from 600 to 1400 μm.

Curve-Fitting Procedure

Points were read from the records of $P(0, t)$ with a low power microscope. Fitting the measured $P(0, t)$ points by the solutions of the diffusion equation (Eq. 1) was accomplished with a digital computer, via a nonlinear least squares curve-fitting routine (Brown and Dennis, 1970).

RESULTS

Validity of Diffusion Equation and Calculation of Diffusion Coefficient

METHOD I   If the one-dimensional diffusion equation (Eq. 1) is valid in the resting sartorius, with Q independent of P, then after the closing of the lower surface of the muscle by the electrode, the kinetics of change in the Po_2 profile within the muscle should be given by:

$$P(x, t) = P_0 - \frac{Q}{2D\alpha}(l^2 - x^2) + \frac{Q}{2D\alpha} \cdot \frac{8}{\pi^2}$$

$$\cdot \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n + 1)^2} e^{-(2n+1)^2 \frac{\pi^2 D \cdot t}{4l^2}} \cdot \sin \frac{(2n + 1)(l - x)\pi}{2l}, \tag{2}$$

where l is the muscle thickness above the platinum cathode, and the other symbols are as defined above (cf. Eq. 1). The derivation of this equation is given by Takahashi et al. (1966). At the muscle surface, the predicted kinetics of Po_2 are thus:

$$P(0, t) = P_0 - \frac{Ql^2}{2D\alpha} + \frac{Q}{2D\alpha} \cdot \frac{8}{\pi^2} \cdot \sum_{n=0}^{\infty} \frac{1}{(2n + 1)^2} e^{-(2n+1)^2 \frac{\pi^2 D \cdot t}{4l^2}}. \tag{3}$$

This equation is potentially useful, because after a relatively short time, all terms

in the infinite series other than the first can be ignored, and the predicted $P(0, t)$ becomes a simple exponential, with rate constant proportional to D. This will occur when $\pi^2 Dt/4l^2 \simeq 0.5$, or for $D = (3-6) \times 10^{-4}$ cm^2/min (Hill, 1966) and $l = 0.7-1.0$ mm, after ~100-400 s.

Fig. 2 shows typical records from experiments of method I at 22.6, 10, and 0°C. Experiments at 22-23° and 10°C were performed in air. At 0°C in air, the changes in surface P o_2 were quite small relative to the starting value; to magnify the changes, experiments were performed in an environment of 7.3% O_2, 2.8% CO_2, and the balance N_2; even then, the changes in Po_2 were still relatively small. In 10-20% of the experiments, the Po_2 at the muscle surface never reached a steady level, but showed a continuous slow decrease; this was
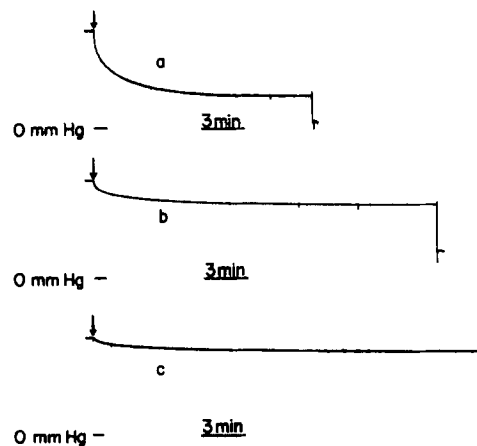

FIGURE 2.   Photograph of typical recordings of the time-course of oxygen electrode current during method I experiments at (a) 22.6°C, (b), 10°C and (c) 0°C. Before the time designated by the arrow, the muscle is oxygenated from both surfaces, and the electrode measures the Po_2 of the chamber gas (153.8, 155.3, and 54.6 mm Hg, respectively, in a, b, and c). At the arrow, the electrode is brought into contact with the lower surface of the muscle.

interpreted as evidence that the rate of oxygen consumption was not constant, and these records were not analyzed.

Fig. 3 shows semilogarithmic plots of $[P(0, t) - P(0, \infty)]$, hereafter referred to as $\Delta P(t)$, for the records displayed in Fig. 2. To fit the measured $P(0, t)$ by Eq. 3, the best-fitting curve of the form ae^{-kt} was calculated for that portion of the measured $\Delta P(t)$ for which Eq. 3 predicted monoexponential kinetics. The lines shown in Fig. 3 were determined by this method, and illustrate average fits. The range of points which were fit by a single exponential were those for which $\Delta P(t)$ was between 45 and 5% of its initial value. The upper limit of this range was set by the condition that the first term of the series of exponentials in Eq. 3 exceed the remaining terms by a factor of 10^3. Points for which $\Delta P(t)$ was <5% of its initial value were not used, because of their relatively large uncertainties.

The fit of a measured $P(0,t)$ by Eq. 3 could also be tested by calculating the best-fitting curve of the form $a \cdot \sum_0^\infty (1/[2n + 1]^2)e^{-(2n+1)^2kt}$ to all points for which

$\Delta P(t)$ was between 100 and 5% of its initial value. This method also produced satisfactory fits, but yielded values for the rate constant k that were, on average, ~15% higher than those obtained from the monoexponential fits. The latter were judged the more reliable, because it was considered that in the vicinity of t = 0, the experimental protocol itself might have caused $P(0, t)$ to differ slightly from the behavior predicted by Eq. 3 (cf. Discussion).

The agreement between the measured $P(0, t)$ and the fitted curves suggests that the one-dimensional diffusion equation for oxygen is indeed valid on the macroscale in the resting sartorius; if so, the measured rate constant k is equal to
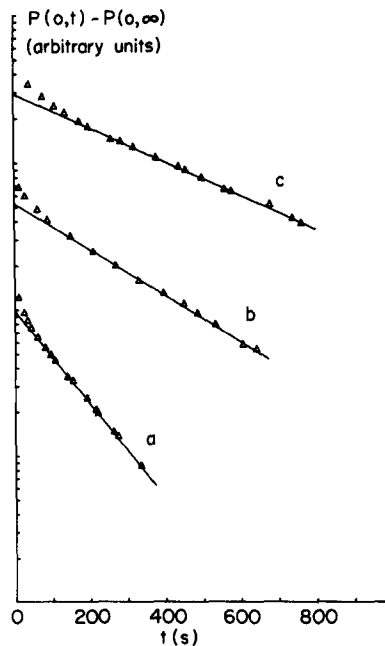

FIGURE 3. Semilogarithmic plots of $[P(0, t) - P(0, \infty)]$ for the records shown in Fig. 2. Solid lines: best-fitting curves of the form ae^{-kt} (See text for the curve-fitting procedure).

$\pi^2 D/4l^2$, and the diffusion constant D is given by:

$$D = 0.405 \, l^2 k. \tag{4}$$

At each temperature, consistent values for D were obtained via Eq. 4, and these are summarized in Table I. As an example of these calculations, for the records shown in Figs. 2 and 3, the values for l were 723, 907, and 768 μm, respectively, at 22.8, 10, and 0°C; the rate constants (cf. Fig. 3) were 0.442, 0.218, and 0.160 min^{-1}, and the values for D calculated from Eq. 4 were thus 9.36, 7.25, and 3.83 × 10^{-4} cm^2/min.

METHOD II Under the conditions of an experiment of method II, after the change in P_{O_2} of the gas phase from P_0 to P_1, the diffusion equation (Eq. 1) predicts that the kinetics of change in the P_{O_2} profile above the platinum cathode will be given by:

$$P(x, t) = P_0 - \frac{Q(l^2 - x^2)}{2D\alpha} + \frac{4(P_1 - P_0)}{\pi}$$

$$\cdot \sum_0^\infty \frac{(-1)^n}{(2n + 1)} \cos \frac{(2n + 1)\pi x}{2l} \left(1 - e^{\frac{-(2n + 1)^2\pi^2 D \cdot t}{4l^2}} \right).$$

(5)

The predicted time-course of P_{O_2} at the lower surface of the muscle is given by:

$$P(0, t) = P_1 - \frac{Ql^2}{2D\alpha} - \frac{4(P_1 - P_0)}{\pi} \cdot \sum_0^\infty \frac{(-1)^n}{(2n + 1)} e^{-(2n+1)^2} \frac{\pi^2 D \cdot t}{4l^2}.$$

(6)

A derivation of Eqs. 5 and 6 is given in Appendix II of Mahler (1976). Eq. 6 is similar to Eq. 3 in that it quickly becomes monoexponential, with rate constant $\pi^2 D/4l^2$; it differs in that the terms of the series of exponents have alternating signs, giving a distinctive S-shape to the early phase of the curve.

Fig. 4 shows results of actual records from experiments of method II at 22.8, 10, and 0°C, and semilogarithmic plots of the same records appear in Fig. 5. To

TABLE I

VALUES OF THE DIFFUSION COEFFICIENT FOR OXYGEN (D) IN THE FROG
SARTORIUS OBTAINED BY EXPERIMENTS OF METHODS I AND II,
VIA EQ. 4

Tempera-ture	$D \times 10^4$					
	Method 1		Method II		Pooled	
°C	n	cm^2/min	n	cm^2/min	n	cm^2/min
22–23	12	8.51±0.36	1	8.81	13	8.54±0.34
10	10	6.36±0.33	1	5.76	11	6.30±0.30
0	12	4.81±0.20	14	5.06±0.24	18	4.94±0.16

quantify the agreement between an observed $P(0, t)$ and that predicted by Eq. 6, the portion of the record for which Eq. 6 predicted monoexponential kinetics was fit by ae^{-kt}. The points fit were those for which $\Delta P(t)$ was between 61 and 5% of its initial value; this range was set by the same criteria explained above for the experiments of method I. These fits were typically excellent. To check that the earlier points in $\Delta P(t)$ were also well described by Eq. 6, the best fitting monoexponentials were used to generate curves of the form $a \cdot \sum_0^\infty (-1)^n/(2n + 1)e^{-(2n+1)^2 k(t-t_D)}$, which according to Eq. 6, should describe the entire time-course of $\Delta P(t)$. The best-fitting time delay t_D was allowed to compensate for the fact that the change in chamber P_{O_2} was slower than a true step; its mean value was ~ 50 s, consistent with the fact that the change in chamber P_{O_2} took ~ 3 min to complete. Fig. 5 shows that these curves did provide satisfactory fits of the early points.

The agreement between the recorded $P(0, t)$ and the general time-course predicted by the diffusion equation again suggests strongly that Eq. 1 is valid in this tissue on the macroscale, and that Eq. 4 can be used to calculate D. The values obtained from the experiments of method II are summarized in Table I. If the diffusion equation is valid, the experiments of methods I and II should yield the same value for D; that this was in fact the case is shown in Table I.

Moreover, experiments of both types were done on eight muscles at 0°C; the mean difference between the paired values for D was 0.07 (±0.38) × 10^{-4} cm^2/min ($P > 0.8$). Table I also gives the values for D obtained by pooling the results from the two types of experiments; in the cases where experiments of both types were done on the same muscle, the average of the two results was used.

If the quantity D calculated from these experiments is a true diffusion coefficient, it should be independent of muscle thickness (l). Regression of D against l showed that this requirement was satisfied: correlation coefficients of 0.43 ($P > 0.15$), 0.22 ($P > 0.5$), and 0.22 ($P > 0.3$) were obtained at 23, 10, and 0°C, respectively (cf. Fig. 6).

Activation Energy for Diffusion of Oxygen in Muscle

On a macroscale, diffusion can be considered a continuous process, and Fick's equation in fact makes this assumption. At the molecular level, however,
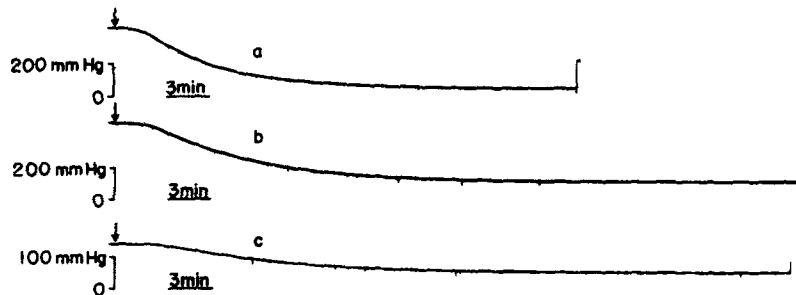

Figure 4. Photograph of typical recordings of the time-course of Po_2 at the lower surface of a muscle during experiments of method II at (a) 22.8°C, (b) 10°C, and (c) 0°C. At the time designated by the arrow, the Po_2 at the upper surface of the muscle was changed from 548 to 154.5 mm Hg in a, from 557 to 155 mm Hg in b, and from 156 to 54.6 mm Hg in c.

diffusion in biological materials proceeds in discrete steps, which occur when the diffusing molecule has sufficient energy to overcome the attractive forces binding it to its neighbors; in a homogeneous medium, this energy has an essentially constant value which is referred to as the activation energy for diffusion, E_A. Diffusion can thus be thought of as a process analogous to a chemical reaction, and by analogy with the Arrhenius equation, it can be expected that the temperature dependence of the diffusion coefficient D will be given by the equation:

$$D_T = D_0 \cdot e^{-E_A/RT}. \tag{7}$$

Fig. 7 shows an Arrhenius plot of D versus T for the pooled results given in Table II. Its linearity makes it reasonable to calculate the value of E_A predicted by Eq. 7 for the diffusion of oxygen in the resting sartorius; this gives the result -3.85 ± 0.06 kcal/mol. Also plotted in Fig. 7 are the values for the diffusion coefficient of oxygen in water reported by Gertz and Loeschke (1954) and Grote (1967) for the temperature range 20–37°C. The corresponding values for E_A are

−3.63 and −3.85 kcal/mol, respectively, and for the pooled data, −3.73 ± 0.13 kcal/mol. Taken together, the data shown in Fig. 7 suggest that (D_{muscle}/D_{H_2O}) is about 0.59 in the frog sartorius. However, although the papers of Gertz and Loeschcke (1954) and Grote (1967) appear to describe the only systematic investigations of the temperature dependence of the diffusion coefficient for O_2 in water, other investigators, each working at a single temperature, have reported somewhat lower values for this parameter. At 25°C, the data of Fig. 7 indicate that $D_{H_2O} \simeq 2.55 \times 10^{-5}$ cm²/s, at the upper extreme of the range of published values tabulated by Goldstick and Fatt (1970), which average 2.2 × 10^{-5} cm²/s. Goldstick and Fatt (1970), using a method similar to that employed
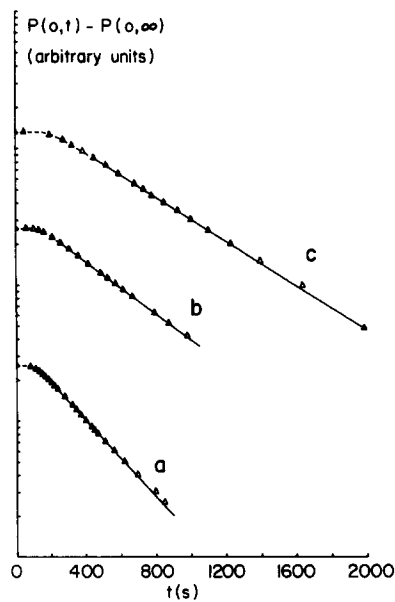

FIGURE 5. Semilogarithmic plots of $[P(0, t) - P(0, \infty)]$ for the records shown in Fig. 4. Solid lines: best-fitting curves of the form ae^{-kt} (See text for curve-fitting procedure). Dashed lines: curves of the form $a \cdot \Sigma_0^\infty (-1)^n/(2n + 1) \exp[-(2n + 1)^2 k(t - t_D)]$; the values of the best-fitting time delay t_D are (a) 78.8 s, (b) 39.4 s, and (c) 35.8 s.

in this paper, reported a value of 2.13×10^{-5} cm²/s. If the latter figures are correct, the value of (D_{muscle}/D_{H_2O}) based on the present results would be about 0.69.

Q_{O_2} in the Resting Sartorius

In the experiments of method I, it follows from Eq. 3 that the rate of oxygen consumption by the muscle is given by:

$$Q = \Delta P \cdot \frac{2D\alpha}{l^2}, \tag{8}$$

where ΔP denotes $P(0, 0) - P(0, \infty)$, the drop in P_{O_2} at the muscle surface

between the initial and final steady states. This relationship has previously been used by Hill (1948) and Kawashiro et al. (1975). Substituting Eq. 4 into Eq. 8 yields a simpler formula which makes use of the nonsteady-state data, and permits the calculation of Q without knowledge of D or l:

$$Q = 0.811 \cdot \Delta P \cdot k\alpha \qquad (9)$$

Given the value of α, the solubility of oxygen in the muscle, the rate of oxygen consumption could be calculated from the data of the present experiments via Eqs. 8 or 9. The average solubility of oxygen in skeletal muscle has apparently never been directly measured, but by using the reasoning of Hill (1966), it can be estimated that α is ~82% as great in the frog sartorius as in isotonic saline. Based on the values of the latter parameter tabulated by Bartels et al. (1971), the
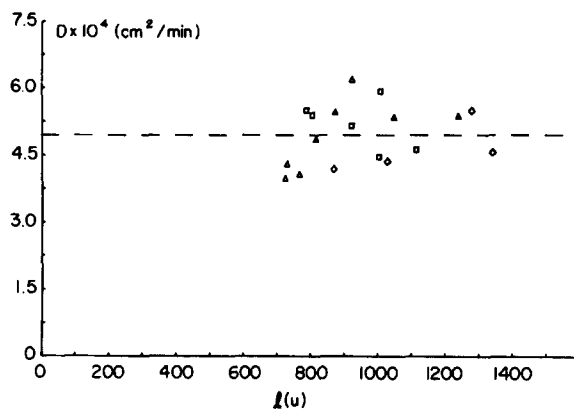

FIGURE 6. Dependence of the diffusion coefficient D on muscle thickness at 0°C. ($\square$) Method I experiment; ($\Diamond$) method II experiment; ($\triangle$) average from method I and method II experiments. Dashed line: $D = 4.94 \times 10^{-4}$ cm^2/min, the mean value at 0°C. The actual line of best fit is $Y = (4.17 \pm 0.84) + (0.00081 \pm 0.00086)X$.

actual values for α assumed in this paper, expressed in μl O_2/g muscle·mmHg, are at 22.6, 10, and 0°C, respectively, 0.0292, 0.0378, and 0.0488.

Given these values, the rates of oxygen consumption by the resting sartorius at 22–23, 10, and 0°C have been calculated from Eq. 9, with the results listed in Table II. Between 10 and 0°C, a fourfold drop in Q_{O_2} was observed (cf. Fig. 8). The method I experiments at 22–23° and 10°C were done in air, whereas those at 0°C were performed in an atmosphere of 7.3% O_2, 2.8% CO_2. This raises the question of whether the gas concentrations per se could have affected the Q_{O_2}. Several lines of evidence suggest that this did not occur. In the first place, it should be noted that in all cases, muscles were well oxygenated: during the method I experiments, the PO_2 at the closed lower surface of the muscle was always lower than anywhere else in the tissue; this was the point at which PO_2 was being measured by the oxygen electrode, and at all temperatures, this minimal PO_2 was safely above the critical PO_2 measured in this muscle (Hill, 1948; Gore and Whalen, 1968; cf. Fig. 2). Secondly, in the experiments of method II, the gas in the chamber was usually changed at $t = 0$ from air to the 7.3% O_2

mixture; if Q had changed markedly because of the change in gas composition, the recorded $P(0, t)$ could not have agreed so strikingly with that predicted by Eq. 6, which assumes that Q is constant. Finally, in three cases at 10° and 15°C, method I experiments were performed on the same muscle in both air and the 7.3% O_2 gas, and the values for Q obtained in the two different gas environments agreed to within 10%.

Calculation of the Permeation Constant K

Using the values of D given in Table I, together with the values of α assumed above for the calculation of Q, the permeation constant K for oxygen in the excised frog sartorius can be calculated as $D\alpha$. These values are listed in Table III.
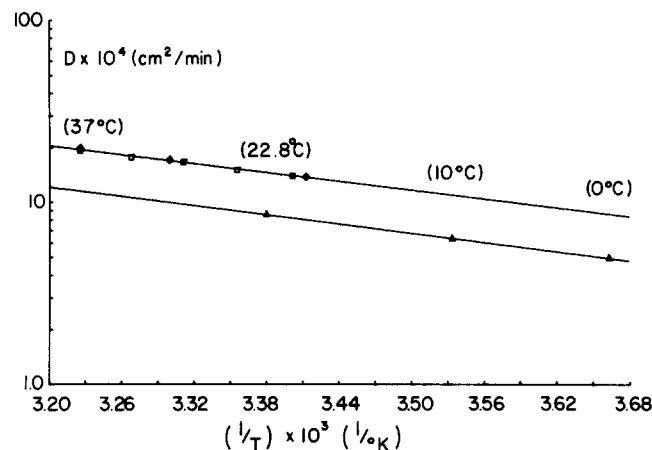

FIGURE 7. Arrhenius plots of the diffusion coefficient D. ($\triangle$) Data of the present study. ($\square$, $\diamond$) Values for D in water reported by Gertz and Loeschcke (1954) and Grote (1967), respectively. Solid lines: lines of best fit in semilogarithmic coordinates. The corresponding values of E_A are -3.73 ± 0.13 kcal/mol and -3.85 ± 0.06 kcal/mol for the upper and lower line, respectively.

DISCUSSION

Methodology

For the method I experiments, it was assumed that the initial P_{O_2} profile above the platinum cathode was given by the steady-state solution of Eq. 1 for a tissue exposed at both surfaces to a constant P_{O_2}. However, these boundary conditions applied only to the portion of the muscle which lay above the recessed electrode; the remainder of the muscle, which rested on the chamber floor, had the boundary condition $(\partial P/\partial x) = 0$, and the initial P_{O_2} profile there was thus quite different from that in the section of the muscle above the electrode. For the approach of method I to be valid, the distance from the platinum cathode to the edge of the shaft, which is about 5 mm, must be large enough to ensure that the P_{O_2} profile above the cathode was essentially unaffected by the "end conditions." Three arguments indicate that this condition was satisfied. First, a rough

indication is furnished by the fact that for a species with a diffusion coefficient on the order of 5×10^{-4} cm²/min, 5 mm is a formidable distance; for instance, if a step change is made at one surface of a 5-mm slab, it will be 90% complete at the other surface only after 500 min. Second, Eq. 3, which is based on the assumption that end effects are neglible, provides a good fit of the observed $P(0, t)$. Finally, the method I experiments yield the same value for D as those of

TABLE II

RATE OF OXYGEN CONSUMPTION (Q_{O_2}) IN THE RESTING
FROG SARTORIUS CALCULATED VIA EQ. 9

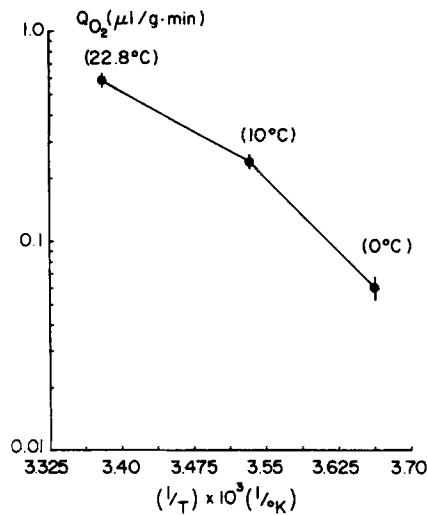| Temperature		Q_{O_2}
°C	n	$\mu l\ O_2/g \cdot min$
22–23	22	0.590±0.042
		(range 0.305–1.11)
10	16	0.241±0.019
		(range 0.094–0.379)
0	14	0.060±.0075
		(range 0.033–0.145)

FIGURE 8.   Arrhenius plot of the resting rate of oxygen consumption. Bars denote ± 1 SEM.

method II, for which the entire lower surface of the muscle does have the same boundary condition, and the problem outlined above does not arise. A comparison of Figs. 3 and 5 shows that the fit of the measured $P(0, t)$ by Eq. 6 is somewhat better than by Eq. 3. Method II thus appears to be the one of choice for testing the validity of Fick's equation (Eq. 1) in an excised tissue.

An objection relevant to the experiments of both types is that the theoretical equations are those for an infinite plane sheet, while the isolated sartorius of *R. pipiens* certainly shows deviations from this configuration. Above the platinum cathode, the measured muscle thickness (*l*) was essentially constant along the longitudinal axis, but typically varied by 30–100 μm within a 400 μm range in

the lateral direction. It is assumed that $P(x, t)$ above this point is the same as in an infinite sheet with the same thickness. Again, perhaps the strongest justification for this is simply that the observed kinetics of P_{O_2} at the muscle surface were well fit by Eqs. 3 and 6, which are, in effect, based on this assumption. This agreement is seen whether (method II) or not (method I) the boundary condition beyond the circumference of the electrode is the same as that within it; this indicates that $P(0, t)$ is the same as would be observed in an infinitely long muscle. As for the effect on $P(x, t)$ of the variation in l in the lateral direction, no additional direct evidence can be offered; it is plausible, though, that the variation in l is sufficiently gradual that at each thickness, the deviation from the P_{O_2} profiles predicted by Eqs. 3 and 6 are acceptably small.

Validity of the Diffusion Equation for Oxygen; the Values of D and K

The first purpose of these experiments was to test the hypothesis that the distribution of O_2 within the excised sartorius of *R. pipiens* is well described by the one-dimensional diffusion equation (Eq. 1). It might be expected that this

TABLE III

VALUES FOR THE PERMEATION CONSTANT $K = D\alpha$ FOR OXYGEN IN THE EXCISED FROG SARTORIUS

Temperature	$D \times 10^4$	α	$K \times 10^6$
°C	cm^2/min	$ml\ O_2/cm^3 \cdot atm$	$ml\ O_2/cm \cdot min \cdot atm$
37	(11.49)	0.01885	2.17
22.8	8.54±0.34	0.02344	2.00
10	6.30±0.30	0.03046	1.92
0	4.94±0.16	0.03933	1.94

It has been assumed that α in muscle is 0.82 times the value in 0.12 M saline. The value of D at 37° was obtained by extrapolation from 0°–22.8° (cf. Fig. 7).

could not be the case: within sufficiently small, uniform volume elements, the diffusion of oxygen in muscle must certainly be well described by the three-dimensional version of Eq. 1; on such a scale it is equally certain, however, that the values of D, α, and Q vary widely within the muscle. For example:

(a) The oxygen consumption rate Q is presumably negligible except at mitochondria;

(b) Myofibrils, which account for ~15% of the volume of a muscle fiber, can apparently be considered fixed structures impermeable to oxygen, so that D, α, and P in this volume are essentially zero;

(c) The cell membrane of the fiber is not a homogeneous structure, and even if it can be considered so on a macroscopic level, at least with respect to the diffusion of oxygen, its composition is sufficiently different from that of the fiber interior that it may present a relative barrier to the passage of oxygen;

(d) In contrast, within the extracellular space, which comprises roughly 10–25% of the muscle volume in an amphibian sartorius (Tasker et al., 1959; Neville and White, 1978) and can presumably be regarded as a dilute aqueous solution of small molecules, it can be expected that D and α will be greater than they are elsewhere in the muscle;

(*e*) The presence of an unsaturated tissue oxygen carrier, such as myoglobin, would cause the P_{O_2} profile to differ from that predicted by Eq. 1 (Wittenberg, 1970; Longmuir et al., 1971. However, Keilin (1925) reported that frog skeletal muscle was "completely devoid" of myoglobin). Additional complications are certainly present, and it is by no means obvious that the distribution of oxygen within an excised muscle can be well approximated by Eq. 1 if boundary conditions are specified only for the tissue surfaces, and average values assumed for D, α, and Q. Fick's law is certainly not generally valid on the macroscale in the heterogeneous media (Barrier, 1968).

On the other hand, this simplification is not necessarily invalid in the present case on account of the inhomogeneities mentioned above. Wang (1954) showed on both theoretical and experimental grounds that diffusion of water in an aqueous protein solution obeyed Fick's law, but that the apparent diffusion coefficient, when compared with that in pure water, was reduced in a predictable manner depending on the concentration, orientation, and water binding capacity of the protein molecules. This suggests that muscle proteins might have a similar effect on diffusion within the interior of a muscle fiber. Kushmerick and Podolsky (1969), Hinke et al. (1973), and Caillé and Hinke (1974) have in fact shown that the movement of a variety of radioactivity labeled substances down the longitudinal axis of a single muscle fiber is well described by Eq. 1, and as pointed out by Caillé and Hinke, their diffusion coefficients appear to be in good agreement with Wang's theory. Based on these results, the concentration profile for oxygen within a single fiber might also be expected to obey Fick's law on the macroscale. Moreover, it is also plausible that the diffusion coefficient for oxygen within muscle cell membranes is similar to that in myoplasm, as suggested by the results of Fischkoff and Vanderkooi (1975), and that the effect of the extracellular space on the distribution of oxygen in muscle can be approximated as a uniform dilution; taken together, these observations make it plausible a priori that, to a good approximation, Fick's equation for the diffusion of oxygen will be valid on the macroscale for an entire excised muscle.

The results presented above suggest strongly that this is indeed the case in the sartorius of *R. pipiens;* during transient changes in the P_{O_2} profile of the muscle, the measured kinetics of P_{O_2} at its surface were in good agreement with detailed predictions based on Eq. 1. However, during the first few minutes after the boundary changes of methods I and II, the recorded kinetics of surface P_{O_2} did deviate somewhat from the solutions of Eq. 1 given by Eqs. 3 and 6, respectively. This may simply mean that the diffusion equation is not precisely valid on the macroscale. However, a plausible alternative explanation is that the initial and (or) boundary conditions assumed in the derivation of Eqs. 3 and 6 were not exactly matched experimentally. In the method II experiments, the recorded $P(0, t)$ changed more slowly than that predicted by Eq. 6; however, in the derivation of Eq. 6, it is assumed that the change in P_{O_2} at the upper boundary of the muscle is made instantaneously, whereas in practice, this took 2–3 min (cf. Methods). If the boundary change was approximated as a step occurring at roughly $t = 50$ s, the recorded $P(0, t)$ was then in excellent agreement with the corresponding solution of Eq. 1 (cf. Fig. 5). In the case of the method I experiments, after the oxygen electrode was brought into contact with the lower

surface of the muscle, the early changes in Po_2 at that surface again appeared to be somewhat slower than expected: if the exponential rate constant $k = \pi^2 D/4l^2$ predicted by Eq. 3 was determined from the latter portion of the recorded curve, and then used to generate the higher order exponential terms called for by Eq. 3, the theoretical $P(0, t)$ usually fell below the recorded curve for the first 1-2 min. The experimental protocol itself may have been at least partly responsible for this. Placing the electrode against the muscle caused a rapid change in its current which did not appear to reflect the actual time-course of surface Po_2 (cf. Methods); this effect was assumed to be restricted to the first few seconds after contact was made, but a smaller, longer lasting discrepancy between electrode current and surface Po_2 might have occurred. Furthermore, in the derivation of Eq. 3, it was assumed that the Po_2 profile within the muscle is constant at $t = 0$; in practice, this condition might not have been exactly satisfied. (In either case, however, the diffusion equation predicts that $P(0, t)$ should eventually become monoexponential, provided Q is constant). From an overall viewpoint, the deviations cited above are relatively small, and even in the worst possible case, it appears that the solution of the one-dimensional diffusion equation (Eq. 1) still provides a good approximation to the Po_2 profile within the excised frog sartorius. This conclusion was anticipated by the work of Gore and Whalen (1968) and Kawashiro et al. (1975), which suggests that Eq. 1 is valid during steady states in excised frog and rat skeletal muscle.

As discussed above, the data reported here imply that the value of D in the excised frog sartorius is ~59-69% as great as in water. Within the extracellular space, D presumably approximates its value in water. If this space constitutes 10-25% of the muscle volume, the present results would then imply a value of 0.44-0.66 for $D_{myoplasm}/D_{H_2O}$, in the same range as the relative values reported by Kushmerick and Podolsky (1969), Hinke et al. (1973), and Caillé and Hinke (1974) for a variety of other small molecules.

Taken together with the theory of Wang (1954), the present results also suggest why the diffusion equation is valid on the macroscale in the excised frog sartorius. In the first place, the activation energy for diffusion of oxygen in the excised sartorius is −3.85 kcal/mol, essentially the same as that reported for the diffusion of oxygen in water by Gertz and Loeschcke (1954) and Grote (1967). The simplest explanation for this seems to be that the molecular level forces impeding the movement of oxygen through muscle membranes are not markedly different from those in the extracellular space and the myoplasm, which can themselves apparently both be considered as dilute aqueous solutions in the present context. Evidence consistent with this conclusion has already been reported by Fischkoff and Vanderkooi (1975), who found that in artificial phospholipid membranes and erythrocyte plasma membranes, the diffusion coefficients for oxygen were on the order of 10^{-5} cm^2/s at 37°C, and the values of E_A averaged about −2.5 kcal/mol. Despite the similar values of E_A in water and muscle, the diffusion coefficient D in the excised sartorius is apparently only ~59-69% as great as in water. Wang (1954) presented a framework within which this result can be interpreted, by providing evidence that the self-diffusion of water within an aqueous protein solution is well described on the

macroscale by Fick's equation, but that D is reduced to a predictable fraction of its value in pure water. As pointed out by Caillé and Hinke (1974), Wang's theory can be applied to the diffusion of any substance in a complex system having obstructing molecules with sequestration sites for the diffusant; in general, the values of D in the complex system and in the pure solvent will be related by:

$$D^{\bullet} = D^{\circ}(1 - f)(1 - \beta\phi), \qquad (10)$$

where $D^{\bullet}$ and D° are the diffusion coefficients in the complex system and the solvent, respectively; ϕ is the volume fraction occupied by obstructing molecules; β is a constant determined by the average shape and orientation of the obstructing molecules; and f is the fraction of the diffusing molecules sequestered by the obstructing species. If the frog sartorius is simply treated as a protein solution, the parameters ϕ, β, and f can be estimated to have values of roughly 0.15–0.30 (Hill, 1930, 1966; Blinks, 1965; Caillé and Hinke, 1974; Palmer and Guliati, 1976), 1.6–1.8 (Wang, 1954; Mahler, 1976), and 0. The range of values for $(D^{\bullet}/D^{\circ})$ predicted on the basis of these assumptions is thus 0.47–0.76, which brackets the range of plausible values based on actual measurements (0.59–0.69). While admittedly imprecise, this analysis suggests the possibility that, at least with respect to the diffusion of oxygen, as a useful first approximation the excised frog sartorius can be considered simply as a protein solution.

The diffusion coefficient for oxygen in skeletal muscle has apparently not been previously measured directly. The values calculated by Hill (1966) are 5.5 $\times$ 10^{-4} cm^2/min at 20°C, and 2.75 $\times$ 10^{-4} cm^2/min at 0°C. At these temperatures, the results of the present study are 8.03 ($\pm$0.32) $\times$ 10^{-4} cm^2/min (interpolated values) and 4.94 ($\pm$0.16) $\times$ 10^{-4} cm^2/min, respectively, and thus differ from Hill's values at a high level of probability ($P < 0.001$). Hill's values at 20°C and 0°C are only $\sim$40–48% and 33–39% as large, respectively, as the corresponding values for D in water; both ratios appear to be below the range of values of $(D_{\text{myoplasm}}/D_{\text{H}_2\text{O}})$ for other small nonelectrolytes, or that predicted by the model of Wang. Hill calculated D as K/α, using the results of Krogh (1919) for K. Krogh's K for 20°C is subject to considerable uncertainty: it is based on a very small sample size; the values of K were proportional to the tissue thickness, which may have been underestimated by Krogh's methods; the thickness of the connective tissue layers surrounding the muscle was apparently not measured in the individual cases; and the value of K in the connective tissue was itself rather uncertain. The values for α assumed by Hill might also be questioned. Finally, Krogh's measurements of K in muscle were all made at 16–20°C, so it seems justified to consider the value assumed by Hill for 0°C to be only a first approximation. Grote and Thews (1962), using an experimental design similar to that employed here, measured D in sections of nonrespiring rat myocardium at 20°C. Their result, as corrected by Grote (1967), was 8.10 $\times$ 10^{-4} cm^2/min, virtually identical with that reported here for the frog sartorius.

The permeation constant K was not measured in the present experiments, but given the assumed values of α, can be calculated as $D\alpha$. These calculated values are roughly 40% higher than those reported by Krogh (1919) and Gore

and Whalen (1968), but ~15% lower than that measured by Kawashiro et al. (1975). However, although exact statistical comparisons are not possible, the 95% confidence limits calculated from the data of Krogh (1.08–1.89 × 10^{-5} ml/ cm·min·atm), Gore and Whalen (0.98–1.86 × 10^{-5} ml/cm·min·atm), and Kawashiro et al. (2.09–2.96 × 10^{-5} ml/cm·min·atm) indicate that the present values of K are reasonably consistent with this previous work (cf. Table III).

The values of K calculated here show a much less marked temperature dependence over the range 0–37°C than predicted by the general formula of Krogh (1919), which was based on a small number of measurements in dog peritoneal membranes, and can be quantified at $(K_T/K_{20}) = (T + 80)/100$, where T is the temperature in °C. On the other hand, because the values for D and α used in the present calculations were both proportional to the values in water, the calculated temperature dependence of K in muscle is the same, in relative terms, as that in water, which manifestly does not obey Krogh's formula. It should be noted that, according to the Wang equation (Eq. 10), the assumption that $(\alpha_{muscle}/\alpha_{H_2O})$ is independent of temperature appears consistent with the parallel linear Arrhenius plots of D in muscle and H_2O. When $(\alpha_{muscle}/\alpha_{H_2O})$ is constant, so presumably are the solvent volume for O_2, the complementary excluded volume ϕ in Eq. 10, and thus (D_{muscle}/D_{H_2O}). By the same reasoning, for any tissue in which the diffusion equation for oxygen is valid on the macroscale, and the solvent volume for O_2 does not vary markedly with temperature, the prediction that (K_{tissue}/K_{H_2O}) is constant thus constitutes a plausible alternative to Krogh's general formula.

Q_{O_2} of the Resting Sartorius

At 22–23°C, the resting Q_{O_2} obtained with the present method is in good agreement with the classical values tabulated by Hill (1966). The relative decrease in Q_{O_2} from 22.8° to 10°C, corresponding to a Q_{10} of 2.0, agrees well with previous work on the temperature dependence of the resting metabolic rate in both intact organisms and isolated tissues, over their physiological temperature ranges (Hill, 1911; Hutchison, 1971; Allen and Kushmerick, 1975). It is thus rather surprising that between 10 and 0°C, the resting Q_{O_2} measured in the present study decreased by a factor of 4.0. The only previously published systematic measurement of the resting Q_{O_2} in the frog sartorius at 0°C appears to be that of Kushmerick and Paul (1976); their value, 0.164 ± 0.027 μl/g·min, is about three times as large as the one reported here. However, the latter value, if correct, might be too small to be measured by the method of Kushmerick and Paul (1976): for a 60-mg muscle, a Q_{O_2} of 0.06 μl/g·min gives an absolute rate of 0.0036 μl/min; relative to the amount of O_2 in their chamber, this represents a decrease of only about 0.5% per h.

In a resting muscle with constant Q_{O_2}, the rate of ATP production via oxidative metabolism is presumably virtually identical to the rate of ATP utilization. If the overall $P:O_2$ for oxidative metabolism does not vary with temperature, the temperature dependence of the resting Q_{O_2} should thus be the same as that for the overall rate of ATP splitting. The latter should simply be an average of the temperature profiles of the various muscle ATPases, weighted by

their relative rates. In this context, it is of interest that the Arrhenius plot for Q_{O_2}, shown as Fig. 8, is strikingly similar to that for the rate of Mg-activated myofibrillar ATPase of frog muscle published by Barany (1967), for the ionic strength nearest the physiological range; for ionic strength 0.064, the Q_{10} between 30 and 10°C was 2.0, whereas between 10 and 0°C, it was 4.2. For actin-activated myosin ATPase of frog muscle, Barany reported somewhat higher Q_{10}'s, but again, the value between 10 and 0°C was more than twice as great as between 20° and 10°C. Similar nonlinear Arrhenius plots, with "break points" occurring around 15°C, have been reported for myofibrillar and actomyosin ATPase of a number of other types of muscle (Levy et al., 1959, Bendall, 1964). In contrast, linear Arrhenius plots have been reported for the Ca^{++}-activated myosin ATPases of frog, tortoise, cat, and sloth skeletal muscle (Barany, 1967), and the sarcolemmal Na^+, K^+-activated ATPase of rabbit skeletal muscle (Boldyrev et al., 1974), with the corresponding Q_{10}'s averaging 2.0–2.5. A plausible explanation for the temperature profile reported here for the resting Q_{O_2} of the sartorius of *R. pipiens* is that the basal rate of ATP utilization in this muscle is primarily attributable to the actomyosin ATPase.

Solubility of Oxygen in Muscle

The values for Q_{O_2} and K reported here are proportional to the values assumed for α, the solubility of O_2 in the excised frog sartorius. Following the reasoning of Hill (1966), it has been assumed that α is 0.82 times the solubility of O_2 in isotonic saline. Hill postulated that: (a) 97% of muscle water, comprising 82% of the volume of the sartorius, is "free" to dissolve O_2 (Hill, 1930); (b) the solubility of O_2 in this fraction is the same as in normal water; and (c) the solubility of O_2 in the remaining tissue volume is negligible. Assumption (a) is controversial. The existence of a fraction of muscle water, which is "bound" to protein and excluded from the solvent volume, has long been postulated (Hill, 1930), and some recent work has indicated that such a fraction may comprise as much as 15–25% of fiber water (Blinks, 1965; Caillé and Hinke, 1974). Other recent evidence, however, suggests that no more than 1% of fiber water is excluded from the solvent volume (Palmer and Guliati, 1976; cf. also Levin et al., 1976). Assumptions (b) and (c) can also be questioned. For example, intrafiber water, even if not "icelike," may nevertheless have reduced mobility in comparison with pure water (e.g., cf. Hazlewood et al., 1974); by analogy with the increase in the solubility of gases which occurs when water mobility is lowered with temperature, intrafiber water might possibly dissolve more O_2 than normal water. Finally, the amount of O_2 dissolved in the nonaqueous volume of muscle might not be negligible; for instance, inasmuch as the olive oil/water partition coefficient for O_2 is about 4 (Battino et al., 1968), the lipid volume of the frog sartorius might contain a significant amount of O_2.

Two lines of evidence, however, suggest that the values assumed for α are substantially correct. First, Grote and Thews (1962) measured α indirectly in sections of nonrespiring rat myocardium at 20°C, by measuring both D and K, and calculating α as K/D. Their results, as corrected by Grote (1967), indicate that $\alpha_{muscle}/\alpha_{H_2O} \simeq 0.84$. Second, the values reported here for Q_{O_2} and K, which

are proportional to the assumed values of α, are in satisfactory agreement with previously published results obtained by direct methods. Values of about 0.5 $\mu l/$ g·min for the resting Q_{O_2} of the frog sartorius at 20°C have been reported by Fenn and Latchford (1932), Gemmill (1934, 1935), and Stannard (1939), and a similar figure can be calculated from the resting rate of heat production given by Hill and Howarth (1957); interpolation based on the data of Table II yields a value of 0.48 ± 0.03 $\mu l/$g·min at 20°C. As discussed above, the values of K calculated here fall within the range of directly measured values reported by Krogh (1919), Gore and Whalen (1968), and Kawashiro et al. (1975).

ADDENDUM.

During the review of this paper, it was pointed out that the respiratory rate Q can be measured with method II experiments, as well as by method I, via Eq. 8 or 9. To do this, it is necessary to calculate ΔP, the steady-state P_{O_2} difference across the muscle. The following equations can be used:

$$\Delta P = P_g - P(0, \infty), \tag{11}$$

$$C_{ss} = a \cdot P(0, \infty) \tag{12}$$

$$= a \cdot P_g - a \cdot \Delta P \tag{12.1}$$

where P_g and $P(0, \infty)$ denote the P_{O_2} of the gas phase and at the lower surface of the muscle, respectively; C_{ss} is the steady-state electrode current (or more precisely, the corresponding chart reading); and a is the calibration constant relating P_{O_2} to chart reading when muscle is the medium external to the electrode (cf. Methods; note that Eq. 12 assumes that the electrode "zero current" is electronically or graphically set equal to zero). As long as the diffusion equation Eq. 1 is valid, ΔP will be constant, and according to Eq. 12.1, a plot of C_{ss} (measured) vs. P_g (known) will be linear, with X-intercept ΔP. n successive method II experiments will provide $(n + 1)$ pairs of values of P_g and C_{ss}, and for the graphical determination of ΔP, it would be desirable to have at least four or five points. However, even a single experiment can be expected to provide a useful first approximation to ΔP. The method II experiments of the present series conducted at 22.8 and 10°C yielded estimates of 107 and 65 mm Hg, respectively, for ΔP, and 0.484 and 0.261 μl $O_2/$g·min for Q_{O_2}, consistent with the results obtained with method I (cf. Table II). At 0°C, the results were less satisfactory. This was not unexpected, because ΔP as measured by method I was typically on the order of only 10 mm Hg (e.g., cf. Fig. 2c), so that relatively large errors could occur in the graphical approximation. 11 method II experiments yielded estimates of −3.4–34.4 mm Hg for ΔP, and −0.029–0.113 (mean ± SEM, 0.012 ± 0.013) $\mu l/$g·min for Q_{O_2}. These values are less than those obtained with method I, but do reinforce the conclusion that the resting Q_{O_2} is relatively small at 0°C (cf. Fig. 8).

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